Claims 20-25, 28-32, 35, and 37-40 were rejected in the Second Office Action under 35 U.S.C. 112, second paragraph, as indefinite. In particular, the Second Office Action objected to the use of the phrases "the sequences", "conditions under which" and "the expression control sequence" for insufficient antecedent basis. Furthermore, the Second Office Action maintained that the phrase "conditions under which" also omitted a step. Applicants respectfully submit that the claims as amended address these rejections presented in the Second Office Action. Accordingly, Applicant requests that these rejections be withdrawn.

Claims 25-27, 32-35 and 39 were also rejected under 35 U.S.C. 102(e) as anticipated by US Patent No. 5,695,977 (hereinafter the "'977 patent"). The Office Action stated that the '977 patent teaches a vector comprising an amplification gene sequence, or a nucleic acid which binds an activator protein, and a selectable marker flanked by recombinase target sequences which may have a negative selection marker outside the recombinase target sequences. Additionally, it was stated that the '977 patent teaches that the vector may be inserted adjacent to an endogenous gene and that the vector may be present in a human cell. Lastly, the Office Action said that the reference teaches a process for testing the action of the amplification gene sequence or nucleic acid which binds an activator protein on the expression of an endogenous target gene.

Applicant notes that a similar, but not identical, 102(e) rejection against Claims 20-28, 30-36, and 39 under the '977 patent was withdrawn in the Office Action dated July 17, 2000. The present 102(e) rejection is more specifically targeted towards the

vector claimed in the present application as opposed to the prior rejection, which targeted the process. In the April 3, 2000 Response to the prior 102(e) rejection Applicant successfully advanced that these claims cover several different embodiments of the present invention and that the Applicant failed to see how any of the embodiments of the invention discussed in the present claims were anticipated by the '977 patent.

The '977 patent is directed towards "hot spots" for the integration for an exogenous nucleic acid sequence. The inventor of the '977 patent discovered that nicks could be created at these areas in a fairly controlled manner. The '977 patent merely recites a "laundry list" of possible elements to be used in the art of homologous recombination. The '977 patent never contemplated the arrangement of the present application.

The '977 patent is used to enhance integration of a construct of interest where there was little preference for either a particular or a unique site for the integration. The construct of interest has the consensus sequence and it may or may not have a second sequence which is homologous to a sequence in the target region.

The '977 patent is concerned with the improved efficiency of homologous recombination through the use of a specific recombination consensus sequence. While the '977 patent discusses the production of recombinant proteins by means of homologous recombination; unlike the present application, however, the '977 patent's expression cassette contains a transcribed sequence of interest (i.e., an exogenous gene). This was an essential feature of the patent. There is no discussion in the '977

'977 patent regarding the activation of the endogenous genes as there is in the present application.

The use of recombinase recognition sites as disclosed in the present application is neither anticipated nor rendered obvious. The '977 reference merely pointed out as an example that through recombinase recognition sequence use, other sequences may be introduced into a cell to determine the effect of variation in the genetic sequence or to cells in which one may introduce sequences at a defined site. There is absolutely no suggestion in the '977 patent regarding using the construct as described in the present application. Further, while the '977 patent does mention the insertion of an amplification gene, it did not contain a disclosure of the use of amplification genes in connection with recombinase recognition sequences. Applicant, therefore, submits that the 102(e) rejection is improper because the '977 patent neither contained a disclosure of endogenous gene expression nor the particular arrangement of the vectors claimed in the present application.

Additionally, a major difference between the '977 patent and the present invention is that the '977 patent requires a two step recombination process due to the use of tandem multicopy sequences in the patent. The decisive difference between the '977 patent and the present application is that the recombinase recognition sequences according to the '977 patent are introduced in the form of tandem multicopy sequences. Therefore, Applicants submit that by means of homologous recombination, a product is obtained which does not contain any functional sequence, but only the recombinase recognition sequence in the form of tandem multicopies. The homologous

recombination construct does not contain any functional sequences and these functional sequences have to be introduced in a further independent process step. In contrast, thereto, the construct of the present application contains two site-specific recombination sequences that are not located next to each other. Therefore, the vector of the present invention is structurally different from the vector disclosed in the '977 patent.

Further, it is clearly evident that the two-step process disclosed by the cited prior art is more cumbersome than the process disclosed in the present application. First a homologous recombination has to be carried out followed by a selection of recombinant clones which do not contain any functional active sequence and, thus, a functional selection of these clones is not possible. Following this step, a second independent procedure requires the insertion of a functional sequence, such as an expression control sequence. Without the functional selection for successful homologous recombination, however, it is extremely difficult to select a clone which allows successful insertion of functional sequences.

Applicants submit that the present invention avoids these drawbacks. The claimed methods carries out the homolgous recombination by using a construct which contains both site-specific recombinase recognition sites and a functional sequence, such as a new expression control sequence. Therefore, in one step, a functional selection for desired clones may be obtained.

Finally, Applicants would like to respectfully resubmit that the rejection remains improper under *In re Bond* which requires that a valid anticipatory reference contain the

elements arranged as required by the claims. This contention is further bolstered by the language of MPEP section 2131 in which it is stated that "[t]he elements must be arranged as required by the claim ..." Accordingly, Applicant respectfully submits that this ground of rejection is not well taken and requests that it be withdrawn.

Additionally, the Second Office Action rejected Claims 36-43 under 35 U.S.C. 103(a) as obvious in view of U.S. Patent No. 6,020,144 (hereinafter the "'144 patent") and the Cruz reference. The Office Action stated that Claims 36-43 are directed to a process for obtaining a DHFR-negative mammalian cell by transfecting the cell with a first vector comprising at least one target sequence for a site-specific recombinase, homologous DHFR sequences which flank the recombinase site-specific sequences and an optional positive and/or negative marker gene.

The Office Action has advanced the position that the '144 patent teaches a process for obtaining a DHFR-negative trypanosomal cell by transfecting it with a first vector comprising at least one target sequence for a site-specific recombinase, homologous DHFR DNA sequences which flank the recombinase site-specific sequences and an optional positive and/or negative selection marker gene. It noted that the '144 patent, however, did not teach that the cell was a mammalian cell and pointed to the Cruz reference to teach that a mammalian cell may be used. It was also noted that Cruz teaches the deletion of DHFR and its replacement with a heterologous DHFR in a cell.

The Office Action then contended that it would be obvious to one with skill in the art at the time of filing to combine the processes for obtaining a DHFR-negative

mammalian cell by transfecting the cell with a first vector that contains a target sequence for a site-specific recombinase and homologous DHFR DNA sequences which flank it. The transfection would then result in homologous recombination causing the vector to insert into the cell's genome, thereby producing a DHFR-negative trypanosomal eukaryotic cell. The Office Action explained the motivation for combining these references was simply that both references investigated the replacement of DHFR in a trypanosomal cell with heterologous DHFR.

In Applicant's Response of April 3, 2000, it was noted that the Cruz publication cited by the Office Action describes the inactivation of a DHFR gene in a protozoal cell and that there was no discussion at all of site-specific recombinase and the recognition sequences thereof. Applicant noted that Cruz was concerned with the analysis of gene function in protozoa alone and that it contains no discussion of the preparation of recombinant proteins. Further, Applicant pointed out that the Cruz reference described a method that was only applicable to protozoa. Finally, Applicant amended Claims 36, 37, and 41-43 to restrict the invention of the present application to mammalian cells, thereby insuring that the Cruz reference was irrelevant to the present application.

Applicant would like to reiterate that the Cruz reference actually appears to teach away from the present invention through its limitation to protozoa. Applicant further notes that the process disclosed in Cruz involves two steps (see page 7172-73 wherein two rounds are discussed and page 7174 wherein a requirement is two rounds), as opposed to the one step process of the present application. Furthermore, a significant difference between the prior art and the present invention is that the present invention

contains the entire recombination process in one step which results in a more elegant process.

Upon review of the newly cited '144 reference, Applicant notes that the '144 patent does not contemplate the use of mammalian cells. Further, the '144 patent discloses a transposon technique. Applicant observes that there is no indication in the '144 patent of the use of site-specific recombinases. Applicant therefore requests that this rejection be withdrawn as not well taken.

Finally, Applicant submits that that Cruz describes the production of DHFR-negative double-targeted Leishmania cells and suggests to apply the process described therein to cultured mammalian cells. The '144 patent discloses a method of producing "conditionally defective" unicellular organisms by suitably modifying their genetic make-up. In one such embodiment, the genetic modification comprises homologous recombination. A further embodiment involves genetic modification which comprises the use of a transposon or a discreet genetic element that endures its own maintenance by inserting onto other autonomously maintained genetic elements (see Column 6, lines 35-39).

The '144 patent, however, does not contain even a hint that the use of site-specific recombinase recognition sequences are possible. Further, Applicants respectfully disagree with the Examiner's assertion that site-specific recombinase recognition sequences are "transposons." It is particularly noted that site-specific recombinase sequences do not have the ability to move as a unit in a more or less

random fashion from one genetic locus to another, as is required by the transposons of the '144 reference.

Thus, Applicants submit that this combination of references cannot render the present invention obvious as they fail to teach every aspect of the present invention and because there is no motivation to combine these references to produce the claimed invention. Thus, Applicants request that this rejection be withdrawn as well.

Claims 20-28 and 30-35 were rejected in the Second Office Action as obvious in light of the '977 patent, WO 94/12650 (hereinafter the "650 application"), and US Patent No. 6,130,364 (hereinafter the "'364 patent"). The Office Action stated that the '977 patent teaches the invention as previously described and that it does not teach a vector which comprises at least one nucleic acid which binds an activator sequence which is a heterologous control sequence. It also stated that the '364 patent teaches a process for introducing a vector by homologous recombination adjacent to a target gene. This vector is comprised of at least one nucleic acid which binds an activator protein which is a heterologous control sequence, etc.

The WO '650 application is alleged to teach a process for introducing a vector by homologous recombination adjacent to a target gene, wherein said vector is comprised of at least one nucleic acid which binds an activator protein which is a heterologous control sequence which is flanked by recombinase sites which is in turn flanked by sequences which target the vector to insert by homologous recombination at a site adjacent to a target gene.

The Office Action has taken the position that one of ordinary skill in the art would have found it obvious to combine these references to create the subject matter of the instant invention. While the differences between this reference and the present application have been discussed previously, Applicant would like to note that while the '977 patent points out that by using recombinase recognition sequences, other sequences may be introduced into a cell to determine the effect of variation in the genetic sequence or to proved cells in which one may introduce sequences at a defined site, there is absolutely no suggestion in the '977 patent towards using the construct as claimed in the present application.

Again, Applicant further notes that while the '977 patent does mention the insertion of an amplification gene, it does not contain a disclosure of the use of amplification genes in conjunction with recombinase recognition sequences. Additionally, the '977 patent contained a two-step process as opposed to the one-step recombination process of the present application. Hence, for these reasons and those listed previously, this reference alone is insufficient to render the present application obvious.

Furthermore, the '364 reference also appears to be inadequate to render the present application obvious. While it may disclose an invention similar to that of the present application, Applicant notes that this reference, too, requires a two-step recombination process. Accordingly, it does not teach the requirement of one-step as seen in the present application. Applicant respectfully submits that this reference discloses a very elaborate method for the insertion of recombinase recognition

sequences wherein a two step process is used. In the first step (Claim 1, section (a)), a first lox site is introduced into a murine embryonic stem cell via homologous recombination. It is from this stem cell that a transgenic mouse is produced and it is from this mouse that an antibody producing cell is obtained. It is into this antibody producing cell that a second lox site is introduced via a second vector. There is no indication at all on the one step process according to the invention or the vector suitable for carrying out this method, respectively. Thus, it appears that this reference is also insufficient to render the present application obvious.

Applicant advances that while the WO 94/12650 application does address endogenous gene expression, no mention is made at all regarding the use of site-specific recombinase recognition sequences and the use of the products of the present application, respectively, which are flanked by recombinase recognition sequences. This reference discloses a method for amending gene expression via homologous recombination. Not even a hint can be found in this reference regarding the introduction of site-specific recombinase recognition sites. Accordingly, Applicant submits that this reference alone is also insufficient to render the present invention obvious.

Applicant respectfully submits that a person with ordinary skill in the art would never have combined these references to create the invention of the present application. Applicant submits that such a person would have never used the one step method according to the invention or the vector suitable therefore in contemplating these references. Instead, such a person would have used the two-step process

disclosed in either the '977 or the '364 patents. Applicant submits that even by combining these references, the present invention would not be rendered obvious.

Additionally, all of the cited prior art recites a two-step process. The first step involves the introduction of site-specific recombinase and the second step involves the insertion of functional sequences into the tandem multicopy sequence. In contrast, the claimed process renounces the use of such tandem multicopy sequences, and uses a one-step process, wherein a functional sequence between two recombinase recognition sites is used for the homologous recombination. Applicants submit that the prior art teaches away from this with its insistence upon two-step processes and that the present invention is, subsequently inventive. Accordingly, Applicant respectfully requests that this ground of rejection be withdrawn as well.

Lastly, Claim 29 was rejected in the Second Office Action under 35 U.S.C. 103(a) as obvious in light of the '977 patent, the '650 patent, and the '364 patent in view of WO 97/37012 (hereinafter the "'012 patent"). Applicant respectfully traverses this rejection as well. Applicant notes that the '012 patent discloses only the insertion of a HIF binding nucleic acid sequence via homologous recombination. Accordingly, for the reasons stated above, it does not appear that this reference would lead to the subject matter of the present application, even if it is taken in combination with the prior references. Applicant, therefore, requests that this ground of rejection be withdrawn.

In the event this paper is not timely filed, Applicant hereby petitions for an appropriate extension of time. The fee for this extension may be charged to our Deposit Account No. 01-2300, along with any other fees which may be due with respect to this paper.

Respectfully submitted,

D. Daniel Dzara, II
Registration No. 47,543

ARENT FOX KINTNER PLOTKIN & KAHN, PLLC
1050 Connecticut Avenue, N.W., Suite 400
Washington, D.C. 20036-5339
Tel: (202) 857-6000
Fax: (202) 638-4810

Enclosures: Marked Up Copy of Claims
Petition to Revive

DDD:mnd

MARKED UP COPY OF CLAIMS

20. A process for changing the expression of a nucleic acid sequence which is present endogenously in a eukaryotic cell, the method comprising

(a) transfecting the cell with a vector comprising the following sequences

(i) at least one sequence, which upon expression is capable of changing the expression of the nucleic acid sequence which is present endogenously in the cell, and selected from the group consisting of a heterologous expression control sequence and an amplification gene,

(ii) a sequence encoding a positive selection marker [gene],

(iii) at least two target sequences for a site-specific recombinase flanking the sequences of (i) and (ii), and

(iv) DNA sequences which flank the sequences of (i), (ii) and (iii) and are homologous to a nucleic acid section in the genome of the cell in order to allow a homologous recombination,

(b) culturing the transfected cell under conditions under which a homologous recombination of the vector takes place,

(c) isolating the cell obtained according to step (b), and

(d) expressing the at least one sequence of (i) to thereby change [heterologous expression control sequence under conditions under which] the

expression of the nucleic acid sequence which is present endogenously in the cell [is changed].

23. The process as claimed in claim 20, further comprising, after step (d), cutting the sequences of (i) and (ii) flanked by the site-specific recombinase target sequences out of the genome of the cell by transient activation of a site-specific recombinase that recognizes the target sequences.

24. A vector suitable for homologous recombination, comprising the following sequences

(i) at least one sequence selected from the group consisting of an expression control sequence and an amplification gene each of which upon expression is capable of changing the expression of the nucleic acid sequence which is present endogenously in the cell,

(ii) a sequence encoding a positive selection marker [gene],

(iii) at least two target sequences for a site-specific recombinase flanking the sequences of (i) and (ii), and

(iv) DNA sequences which flank the sequences of (i), (ii) and (iii) and are homologous to a nucleic acid section in the genome of a cell in order to allow a homologous recombination, and

(v) optionally a sequence encoding a negative selection marker [gene].

25. A vector, comprising

(i) at least one sequence selected from the group consisting of a heterologous expression control sequence and an amplification gene each of which upon expression is capable of changing the expression of the nucleic acid sequence which is present endogenously in the cell,

(ii) a sequence encoding a positive selection marker [gene],

(iii) at least two recombinase target sequences flanking the sequences of (i) and (ii), and

(iv) optionally a sequence encoding a negative selection marker [gene].

28. A process for changing the expression of a nucleic acid sequence which is present endogenously in a eukaryotic cell, the method comprising

(a) transfecting the cell with a vector comprising

(i) at least one nucleic acid sequence which binds an activator protein,

(ii) a sequence encoding a positive selection marker [gene], and

(iii) DNA sequences which flank the sequences of (i) and (ii) and are homologous to a nucleic acid section in the genome of the cell in order to allow a homologous recombination,

(b) culturing the transfected cell under conditions under which a homologous recombination of the vector takes place,

(c) isolating the cell obtained according to step (b), and

(d) expressing the sequence of (i) under conditions under which the activator protein is bound thereby changing the expression of the nucleic acid sequence which is present endogenously in the cell [is changed].

32. A vector suitable for homologous recombination, comprising the following sequences

(i) at least one nucleic acid sequence which binds an activator protein,

(ii) a sequence encoding a positive selection marker [gene], and

(iii) DNA sequences which flank the sequences of (i) and (ii) and are homologous to a nucleic acid section in the genome of a cell in order to allow a homologous recombination.

35. A process for testing the influence of non-coding nucleic acid sequences from the region of a target gene present endogenously in a eukaryotic cell on its expression, the process comprising

(a) transfecting the cell with a vector comprising

(i) a heterologous expression control sequence which is active or can be activated in the cell and is operatively linked with a reporter gene, and

(ii) non-coding nucleic acid sequences on the 5'-side and/or the 3'-side from the region of the target gene,

(b) culturing the cell under conditions under which the heterologous expression control sequence is active, and

(c) measuring [the] expression of the reporter gene.

36. A process for obtaining a DHFR-negative eukaryotic cell, the process comprising

(a) transfecting [the] a DHFR-positive cell with a first vector comprising

(i) at least one DHFR-negative target sequence for a site-specific recombinase;

(ii) DNA sequences which flank sequence (i) and are homologous to a DHFR nucleic acid sequence which is present endogenously in the cell in order to allow a homologous recombination,

(iii) optionally a sequence encoding a first positive selection marker [gene], and

(iv) optionally a sequence encoding a negative selection marker [gene],

(b) culturing the transfected cell under conditions under which a homologous recombination of the vector takes place thereby incorporating the DHFR-negative target sequence into the DHFR-positive cell to create a DHFR-negative cell, and

(c) isolating the [cell] cells obtained according to step (b) to obtain a DHFR-negative eukaryotic cell.

37. A process for obtaining a eukaryotic cell containing a nucleic acid sequence to be amplified and a heterologous DHFR gene, the process comprising

(a) obtaining a DHFR-negative eukaryotic cell by the process as claimed in claim 36,

(b) transfecting the cell of step (a) with a second vector comprising

(i) a nucleic acid sequence coding for a DHFR,

(ii) a nucleic acid sequence to be amplified which codes for a protein in an expressible form,

(iii) optionally a sequence encoding a second positive selection marker [gene], and

(iv) at least two recombinase target sequences flanking the sequences of (i), (ii) and (iii), if present,

(c) culturing the transfected cell under conditions under which the sequences of (i), (ii) and (iii), if present, are integrated into the recombinase target sequence that is already present in the genome of the cell, and

(d) isolating the cell obtained according to step (c) to obtain a eukaryotic cell containing a nucleic acid sequence to be amplified and a heterologous DHFR gene.

39. A vector, comprising

(i) a nucleic acid sequence coding for a DHFR,

(ii) a nucleic acid sequence to be amplified which codes for a protein in an expressible form,

(iii) optionally a sequence encoding a positive selection marker [gene], and

(iv) at least two recombinase target sequences flanking the sequences of (i), (ii) and (iii), if present.

40. A vector suitable for homologous recombination, comprising

(i) optionally a sequence encoding a positive selection marker [gene],

(ii) at least one recombinase target sequence which flanks the sequence of (i), if present,

(iii) DNA sequences which flank the sequences of (i), if present, and (ii) and which are homologous to a DHFR nucleic acid sequence which is present endogenously in a cell in order to allow a homologous recombination, and

(iv) optionally a sequence encoding a negative selection marker [gene] which is outside the homologous DNA sequences (iii).